Zoosyst. Evol. 101 (3) 2025, 1137–1154 | DOI 10.3897/zse.101.153548

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# A new super narrow-ranged endemic *Phoxinus* minnow (Leuciscidae) from the Caucasus

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*https://zoobank.org/36725566-D547-47E8-B652-7AA902D77970*

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Academic editor: Nicolas Hubert ♦ **Received** 20 March 2025 ♦ **Accepted** 20 May 2025 ♦ **Published** 25 June 2025

## Abstract

A new species, *Phoxinus ayukensis* **sp. nov.**, from the Kuban basin (Northwestern Caucasus) is described. This species has an extremely narrow range of approximately 5–7 km in a seasonally almost drying stream within the karstic region. The new species has a moderate genetic distance in cytochrome *b* mtDNA (*p*-distance = 0.026 ± 0.005) from geographically and genetically close species in the Kuban basin—*P. adagumicus*—and differs from all known *Phoxinus* species by a highly increased number of scales in the lateral series: 87–105 (mean 97.7), differs from other species from Eastern Europe and the Caucasus (*P. adagumicus*, *P. chrysoprasius*, *P. colchicus*, and *P. isetensis*) by an increased frequency of occurrence of seven rays in the pelvic fin (ca. 35%) and an increased number of scales between bases of dorsal and anal fins (32–41, mean 36.7). Morphologically, the new species is the most different from the other Caucasian species, but it is closer to the Northern minnow *P. isetensis*; that might be explained by phenotypic convergence caused by adaptation to cold waters. The range of the new species is limited by waterfalls in the upper reach of the Ayuk Stream, where subterranean water outlets probably contributed to its survival. The role of Pleistocene glaciations in shaping the modern distribution and environmental differences in evolutionary divergence of Caucasian *Phoxinus* is highlighted. Low genetic diversity coupled with extremely limited range within the seasonally half-drying stream suggests a high rank of protection for new species.

## Key Words

Biodiversity, conservation, freshwater fishes, integrative taxonomy, Kuban Basin, karst springs, locally restricted species

## Introduction

Minnows of genus *Phoxinus* Rafinesque, 1820 are small rheophilic fish of family Leuciscidae Bonaparte, 1835, distributed in northern Eurasia from the Pyrenees to the Pacific Ocean drainage. Taxonomy, initially based on morphology, could not reliably classify species within the genus and was ambiguous. On the one hand, researchers noticed that representatives of the genus in different parts of range have differences, on the other hand, insignificance of these differences and their complex geography did not allow solving the problem of species classification. As a result of uncertainty almost all previously described species were synonymized by Berg (1949) into one species – *Phoxinus phoxinus* (Linnaeus, 1758). Recent genetic studies made it possible to fully or partially confirm species previously described by morphology – e.g., *P. bigerri* Kottelat, 2007, *P. colchicus* Berg, 1910, *P. lumaireul* (Schinz, 1840), *P. karsticus* Bianco & De Bonis, 2015, *P. septimaniae* Kottelat, 2007, *P. marsilii* Heckel, 1836, *P. csikii* Hankó, 1922 (Palandačić et al. 2017), *P. chrysoprasius* (Pallas, 1814) (Bogutskaya et al. 2023), and *P. isetensis* (Georgi, 1775) (Artaev et al. 2024a) as well as to describe new species – *P. krkae* Bogutskaya, Jelić, Vucić, Jelić, Diripasko, Stefanov & Klobučar, 2019 (Bogutskaya et al. 2019), *P. dragarum* Denys, Dettaï, Persat,

Daszkiewicz, Hautecoeur & Keith, 2020, *P. fayollarum* Denys, Dettaï, Persat, Daszkiewicz, Hautecoeur & Keith, 2020 (Denys et al. 2020), *P. abanticus* Turan, Bayçelebi, Özuluğ, Gaygusuz & Aksu, 2023 (Turan et al. 2023), *P. radeki* Bayçelebi, Aksu & Turan, 2024 (Bayçelebi et al. 2024), and *P. adagumicus* Artaev, Turbanov, Bolotovskiy, Gandlin & Levin, 2024 (Artaev et al. 2024b). However, these studies revealed a discordance between genetic and morphological results (Palandačić et al. 2017, 2024).

A recent taxonomic revision of the Caucasus *Phoxinus* minnows revealed two locally distributed species in the Kuban basin. One refers to *P. colchicus* distributed in the Belaya River basin and separated from the main range in east Black Sea drainage. Another recently discovered and described species, *P. adagumicus*, is distributed in the Adagum River basin in the lower Kuban system (Artaev et al. 2024a). Of particular interest is the isolated population of *Phoxinus* from the upper reaches of the Ayuk Stream, the existence of which has been known for over 75 years (Sukhanova and Troitskiy 1949). Some researchers have noted its morphological difference (Otrishko and Emtyl 2013a, 2013b); however, no taxonomic descriptions have been made. In the present study, we use molecular and morphological methods (integrative taxonomy) to clarify the taxonomic status of *Phoxinus* minnow from the upper reaches of the Ayuk (= Burlachenkova Shchel, Peschernaya Shchel, Semenovskaya Shchel) Stream and to describe a new species.

# Materials and methods

## Sampling

Materials for morphological studies and partly for genetic studies were collected in two localities: the main material was sampled above waterfalls on the Ayuk Stream (44.4756°N, 39.0196°E, 19 July 2024), while one individual was sampled in the lower reach near the confluence with the Chepsi River (44.4987°N, 39.0723°E, 18 May 2023). Fish were caught using a frame net with a mesh size of 6–8 mm. Fish were euthanized in a solution of clove oil and photographed in an aquarium with artificial lighting using a Nikon D5300 camera (Nikon Corporation, Tokyo, Japan) with a Nikkor 60 mm f/2.8G lens (Nikon Corporation, Tokyo, Japan) using a physical white swatch for color correction. Fin clips (pectoral or pelvic) were taken from some specimens (DNA vouchers) and placed in 96% ethanol for subsequent DNA extraction in the laboratory. Then most fish were preserved in 10% formalin, while some samples (usually small-sized individuals) were preserved in 96% ethanol for molecular analysis. Formalin-fixed specimens were washed out in running water and transferred to 70% ethanol for long-term storage.

The types (holotype, part of paratypes), additional, and comparative material are deposited in the Fish Collection of the Papanin Institute for Biology of Inland Waters of the Russian Academy of Sciences, Borok, Russia (IBIW_FS); the rest of the paratypes are kept in the Zoological Institute of the Russian Academy of Sciences, Saint Petersburg, Russia (ZISP), and the Zoological Museum of the Moscow University, Moscow, Russia (ZMMU).

## Morphological studies

Morphological data were collected from *Phoxinus* sp. (n = 49) specimens obtained from the Ayuk Stream. In the morphological study, we followed Bogutskaya et al. (2019, 2023) and Artaev et al. (2024b). In particular, 42 morphometric (Suppl. materials 1, 2), 20 meristic, and two qualitative characters (Suppl. materials 1, 3) were processed. Abbreviations of morphometric characters are given in Suppl. material 2. Morphometric measurements were taken from the left side of the body using a digital caliper to the nearest 0.1 mm by one operator for the purposes of consistency, as recommended by Mina et al. (2005). Meristics (except for skeleton) and type of breast scalation (Bogutskaya et al. 2019) were assessed using material stained in an ethanol solution of alizarin red S (Taylor and Van Dyke 1985 with modifications), followed by short exposure to 1–2% potassium hydroxide and preservation in 70% ethanol.

Sex was determined by the shape and size of the pectoral and pelvic fins and their rays (Frost 1943; Berg 1949; Chen 1996; Bogutskaya et al. 2019). External meristics were counted on the left side. Standard length (SL) was measured from the tip of the upper lip to the end of the hypural complex. The total number of pectoral and pelvic-fin rays was counted on the left fins. The last two branched rays articulated on a single pterygiophore in the dorsal and anal fins are counted as one. The rays in the caudal fin were counted from the longest uppermost unbranched principal ray to the longest lowermost unbranched principal ray, inclusive. The average number of procurrent rays was counted as the average value between visible (extending beyond the scaly cover) dorsal and ventral procurrent rays. Scales above the lateral line were counted between the lateral line and base of the first unbranched ray in the dorsal fin; scales below the lateral line were counted between the lateral line and base of the first unbranched ray in the anal fin. In both cases, lateral line scales were not taken into account. The number of scales between the bases of the dorsal and anal fins was counted as the number of scales between the first unbranched rays of the dorsal and anal fins. This character can also be counted as a sum of scales below the lateral line, above the clateral line, and one scale in the lateral line. The number of anterior gill rakers of the first gill arch was counted on the left side of the specimens. The number of scale rows was counted on the left and right breast patches, and an average value was taken. The counts of external meristic characters and assessment of qualitative characters were done using the stereomicroscope MC-2-ZOOM (Micromed, Saint Petersburg, Russia) and digital video monocular microscope YIZHAN 05-SS 48MP with 180 × lens (Yizhan, China). Vertebrae and pterygiophores were counted following Naseka (1996) with modification by Bogutskaya et al. (2019) using radiographs made by

X-ray equipment PRDU II (ELTECH-Med, Saint Petersburg, Russia). Images of pharyngeal teeth were obtained using a JEOL JSM-6510LV scanning electron microscope (Jeol, Tokyo, Japan).

Descriptive statistics were obtained in Microsoft Excel. Comparison of samples of different species (Suppl. material 2) was performed using the Kruskal-Wallis test followed by Dunn's post hoc test with Bonferroni correction [rstatix (Kassambara 2020) and tidyverse (Wickham et al. 2019) packages in R version 4.3.1 (Ihaka and Gentleman 1996)]. Differences between sexes were tested using the Mann-Whitney U test in PAST 4.13 (Hammer and Harper 2001). UPGMA dendrograms based on Euclidean distances of average values of 41 morphometric characters for five *Phoxinus* species were resampled through 10000 bootstrap replicas using Past 4.13 (Hammer and Harper 2001). In particular, first, average values in samples were calculated for each character; then, based on these values, an average for each species was obtained. To evaluate the significance of differences in meristic characters among species, we have performed a principal component analysis (PCA) in PAST 4.13. Subsequently, a MANOVA test was conducted also in Past 4.13 on the five principal components, which accounted for 91.2% of the variance.

For the analysis of external meristic characters, we used eight variables: total number of scales in the lateral series (sql), number of pored lateral-line scales (llt), average number of procurrent rays (AvPR), number of scales between bases of dorsal and anal fins (bda), mean number of scale rows on left and right breast patches (BrScale), total number of rays in pectoral fin (P), total number of rays in ventral fin (V), and number of circumpeduncular scales (cps). The discriminant function analysis (DFA) was performed in Statistica 12 (Statistic for Windows, Statsoft; www.stasoft.com) using standardized data within the same software. The obtained *p*-values were used to assess the significance of differences between groups, and the Mahalanobis distance squared matrix was subsequently used to construct a UPGMA dendrogram, also employing Past 4.13. The same eight external meristic characteristics were used in principal component analysis (PCA), which was performed using the ggfortify package based on standardized data (Tang et al. 2016) in R.

### Molecular analyses

DNA was isolated by salt extraction (Aljanabi and Martinez 1997) from ethanol-fixed fin clips. Two mitochondrial markers were analyzed. The mitochondrial cytochrome *c* oxidase subunit I (COI) barcode region was amplified using an M13-tailed primer cocktail following the PCR protocol from the study of Ivanova et al. (2007). In addition, cytochrome *b* (cyt*b*) fragments were amplified by PCR using the primers GluF and ThrR (Machordom and Doadrio 2001). PCR amplifications were performed using Evrogen ScreenMix-HS under conditions described by Levin et al. (2017).

Sequencing of the PCR products, purified by ethanol and ammonium acetate (3 M) precipitation, was conducted using the Applied Biosystems 3500 DNA sequencer (Thermo Fisher Scientific, USA) in the Core Facility of IBIW RAS. DNA chromatograms were checked for errors in FinchTV 1.4.0 (Rothgänger et al. 2006), and the DNA sequences were aligned using the MUSCLE algorithm in MEGA7 (Kumar et al. 2016). Phylogenetic analysis was performed on COI (567 bp) and cyt*b* (1089 bp) concatenated sequences. In addition to the 12 newly determined COI and cyt*b* sequences (six specimens) in this study (COI: PQ998249–PQ998254 with holotype PQ998252, and cyt*b* PV105506–PV105511 with holotype PV105509), 221 concatenated sequences of all available *Phoxinus* spp. were mined from the GenBank (https://www.ncbi.nlm.nih.gov/). Three outgroups representing the leuciscid genera *Pseudaspius* Dybowski, 1869, *Rhynchocypris* Günther, 1889, and *Oreoleuciscus* Warpachowski, 1889 were selected according to the previous phylogenetic studies (Palandačić et al. 2015, 2020) (Suppl. material 4). Only unique haplotypes were used in downstream phylogenetic analyses. The numbers of some clades that do not have unique species names are given according to the study of Palandačić et al. (2020).

The Bayesian phylogenetic analysis was performed in a Bayesian statistical framework implemented in BEAST v.1.10.4 (Hill and Baele 2019) with $2 \times 10^7$ MCMC generations (10% burn-in) and parameters sampled every 1000 steps. The substitution models by codon position for Bayesian analysis were selected in PartitionFinder v.2.1.1 (Lanfear et al. 2016) with the greedy algorithm (Lanfear et al. 2012) (Suppl. material 5). Maximum likelihood phylogenies were inferred using IQ-TREE v.2.2.0 (Nguyen et al. 2015) in PhyloSuite v1.2.3 (Zhang et al. 2020; Xiang et al. 2023) under the Edgelinked partition model for 1000 ultrafast (Minh et al. 2013) bootstraps. ModelFinder v.2.2.0 (Kalyaanamoorthy et al. 2017) in PhyloSuite v.1.2.3 was used to select the best-fit partition model (edgelinked) using the AICc criterion (Suppl. material 5).

Nucleotide ($\pi$) and haplotype diversity (*Hd*), and the average number of nucleotide differences (*K*) were computed using the DnaSP v.6 (Rozas et al. 2017). The average and maximum intraspecies, as well as the average pairwise interspecies *p*-distance, were calculated using MEGA7 (Kumar et al. 2016) with 1000 bootstrap replicas.

## Results

### Phylogenetic placement and genetic diversity

Phylogenetic Bayesian tree of the genus *Phoxinus* showed that *P. ayukensis* sp. nov. has its own lineage, being sister to *P. adagumicus*, which is also distributed in the Kuban system in the Sea of Azov basin (Artaev et al. 2024b), with high support in both BI and ML analyses (Fig. 1). Both species from the Kuban basin combined together (*P. adagumicus* and *P. ayukensis* sp. nov.)

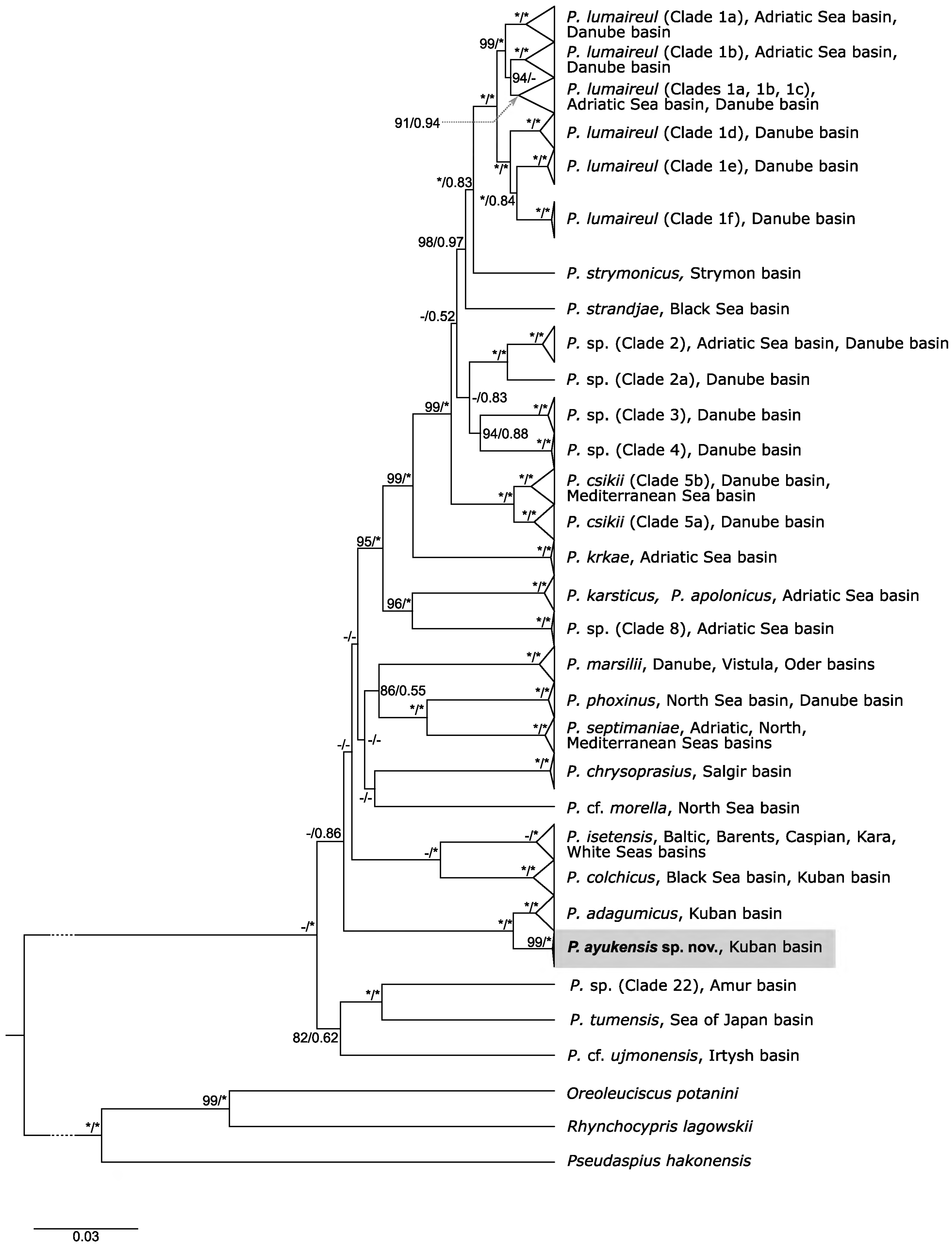


**Figure 1.** BI consensus tree of concatenated COI and cyt*b* mtDNA sequences representing available *Phoxinus* species in the NCBI database combined with our data set. *Phoxinus ayukensis* sp. nov. is highlighted. Bootstrap values/posterior probabilities above 50/0.5 are shown; asterisks represent 100/1 bootstrap/posterior probability values. The scale bar is in expected substitutions per site. The nodes with multiple specimens were collapsed to a triangle, with the horizontal depth indicating the level of divergence within the node.

represent the earliest branching lineage of the *Phoxinus* in Europe, although with moderate (0.86 for BI) or without support (ML). Genetic *p*-distance between *P. ayukensis* sp. nov. and *P. adagumicus* is moderate (0.022 ± .005 in COI+cyt*b* concatenated sequence; 0.026 ± 0.005 in cyt*b* and 0.013 ± 0.005 in COI) but very remarkable given they

**Table 1.** Intraspecific genetic variation of *Phoxinus ayukensis* sp. nov. and *P. adagumicus* on COI+cyt*b* concatenated sequences. *n*/*H* – sample size/number of haplotypes; *Hd* ± SD – haplotype diversity ± standard deviation; *π* ± SD – nucleotide diversity (per site) ± standard deviation; *K* – average number of nucleotide differences; Mean intra-sp ± SE – mean intraspecies *p*-distance ± standard error; Max intra-sp – maximum intraspecies *p*-distance; Distance to NS ± SE – mean *p*-distance to a nearest species ± standard error (based on 1000 bootstrap replicas).

| Species | n/H | Hd ± SD | π ± SD | K | Mean intra-sp ± SE | Max intra-sp | Nearest species | Distance to NS ± SE |
|---|---|---|---|---|---|---|---|---|
| *P. adagumicus* | 12/12 | 1.000 ± 0.034 | 0.0070 ± 0.0009 | 11.7 | 0.0070 ± 0.0011 | 0.013 | *P. ayukensis* sp. nov. | 0.0218 ± 0.0033 |
| *P. ayukensis* sp. nov. | 6/2 | 0.333 ± 0.215 | 0.0002 ± 0.0001 | 0.3 | 0.0002 ± 0.0002 | 0.001 | *P. adagumicus* | 0.0218 ± 0.0033 |
| All | 18/14 | 0.935 ± 0.052 | 0.0133 ± 0.0010 | 22.1 | 0.0133 ± 0.0017 | 0.025 | — | — |

inhabit the same riverine basin. Intraspecies divergence of *P. ayukensis* sp. nov. is very low (0.0002). Genetic diversity (haplotype, nucleotide diversity, etc.) of the sample of *P. ayukensis* sp. nov. is also very low compared to sister species *P. adagumicus* (Table 1).

## Systematics

**Class Actinopterygii Klein, 1885**
**Order Cypriniformes Bleeker, 1859**
**Family Leuciscidae Bonaparte, 1835**
**Genus *Phoxinus* Rafinesque, 1820**

### *Phoxinus ayukensis* sp. nov.

https://zoobank.org/7D819936-2989-4F05-97D9-B2A933BB29A0
Figs 2, 3, 8
English name: Ayuk minnow
Russian name: Аюкский гольян

*Phoxinus phoxinus* – Sukhanova and Troitskiy 1949: 154, 164–165 (part., upstream of Ayuk River); Emtyl et al. 1994: 137–141, figs 1, 2 (part., Ayuk River); Reshetnikov et al. 2003: 301–302 (part., Kuban River).

*Phoxinus phoxinus kubanicum* Emtyl & Ivanenko, 2002, *unavailable name*: 90–92, fig. 69 ex Berg 1949, not *P. adagumicus* and *P. ayukensis* sp. nov. (part., Psekups River basin); Otrishko and Emtyl 2013a: 20 (part., Kuban basin).

*Phoxinus kubanicus* [sic] – Otrishko and Emtyl 2013b: 69–70 (part., Ayuk River).

**Type material.** ***Holotype***, • IBIW_FS_501, female (54.3 mm SL, GenBank accession numbers PQ998252 – COI, PV105509 – cyt*b*), Russia, Krasnodar Krai, Kuban River drainage, Ayuk (= Burlachenkova Shchel, Peshchernaya Shchel, Semenovskaya Shchel) Stream, tributary of Chepsi River, upstream Fanagoriyskoe Vil., 44.4756°N, 39.0196°E, 19 July 2024, O.N. Artaev leg. ***Paratypes***: • 3 females, 1 male (IBIW_FS_502), SL 37.5–54.8 mm; • 2 females, 1 male (ZISP 57075), SL 39.3–50.2 mm; • 3 females (ZMMU P-24653), SL 38.6–50.0 mm, all from the same locality and date as holotype.

**Additional material.** Suppl. material 6.

**Comparative material.** Suppl. material 6.

**Material used in genetics.** Suppl. material 4.

**Diagnosis.** *Phoxinus ayukensis* sp. nov. is distinguished from other European and circum-Black Sea *Phoxinus* species (*P. abanticus*, *P. adagumicus*, *P. bigerri*, *P. chrysoprasius*, *P. colchicus*, *P. csikii* (Clade 5), *P. dragarum*, *P. fayollarum*, *P. isetensis*, *P. karsticus*, *P. krkae*, *P. lumaireul* (Clade 1b, 1a), *P. marsilii* (Clade 9), *P. radeki*, *P. septimaniae*, and *P. strandjae* Drensky, 1926) by increased number of scales in the lateral series 87–105 (mean 97.7); from species from Eastern Europe and the Caucasus (*P. adagumicus*, *P. chrysoprasius*, *P. colchicus* and *P. isetensis*) by increased frequency of occurrence of seven rays in the pelvic fin (ca. 35%) and increased numbers of scales between first unbranched rays of dorsal and anal fins (32–41, mean 36.7).

**Description.** The live and preserved appearance as well as radiograph of holotype is shown on Fig. 2, general appearance of live female and male of *Phoxinus ayukensis* sp. nov. from type locality is shown on Fig. 3, morphometrics of holotype, paratypes and additional material from the type locality with level of significance of sex-related differences are given in Table 2, meristic and qualitative characters for specimens from type locality are given in Table 3, primary morphological data for specimens from the type locality (holotype, paratypes and additional material) are given in Suppl. material 1, morphometrics of *P. ayukensis* sp. nov., *P. isetensis*, *P. adagumicus*, *P. chrysoprasius* and *P. colchicus* with their comparison are given in Suppl. material 2, meristic and qualitative characters of *P. ayukensis* sp. nov. and other *Phoxinus* spp. are given in Suppl. material 3.

***Morphometrics*** (Table 2, Suppl. materials 1, 2). The new species has a rather small size—the maximum SL is 54.8 mm. The new species has eye horizontal diameter 7.0% HL in holotype, 7.0–8.7% in paratypes and additional material from type locality; caudal peduncle width 7.9% SL in holotype, 7.0–8.4% in paratypes and additional material from type locality; head length 145.2% body depth in holotype, 120.2–152.9% in paratypes and additional material from type locality.

***Meristics*** (Table 3, Suppl. material 3). Dorsal fin with 3 unbranched and 7 ½ branched rays. Anal fin with 3 unbranched and 7 ½ branched rays. Pectoral fin with 14–16 rays, often 15–16. Pelvic fins with 7–8 rays. Caudal fin with 19 rays (rarely 18 and 20 rays). Number of dorsal procurrent caudal-fin rays 10–13. Number of ventral procurrent caudal-fin rays 6–10. Pharyngeal teeth are two-rowed, with the common formula 2.5–4.2. However, appr. 30% of individuals exhibit a reduction of the second row to a single tooth, at least on one pharyngeal bone. Total number of vertebrae in the holotype 40, 39–41 in the paratypes, and in the add. material, commonly 40. Number of abdominal vertebrae in the holotype 22, 21–23 in the paratypes, and 21–24 in the add. material, commonly


**Figure 2.** Holotype of *Phoxinus ayukensis* sp. nov. (SL 54.3 mm, IBIW_FS_501, female). **A.** Live appearance; **B.** General appearance of the preserved specimen; **C.** Radiograph.

22 or 23. Number of caudal vertebrae in the holotype 18, 17–19 in the paratypes, and 16–19 in add. material, commonly 17 and 18. Number of predorsal abdominal vertebrae in the holotype 14, 14–15 in the paratypes, and in the add. material, commonly 14 or 15. Number of anal-fin pterygiophores in front of the first caudal vertebrae in the holotype 5, 4–7 in the paratypes, and in the add. material, commonly 5 or 6. Difference in the number of abdominal and caudal vertebrae in the holotype 4, 2–6 in the paratypes, and 2–8 in the add. material, commonly 4–6.

Lateral line incomplete and discontinuous. Total number of scales in the lateral line series 87–105, mean 97.7. Total number of lateral-line (pored) scales 9–54, mean 30.5. Number of scales between bases of dorsal and anal fins 32–41, mean 36.7. Number of circumpeduncular scales 41–49, mean 45.1.

Gill rakers on the first left gill arch 7–9, mean 7.9.

***Qualitative characters.*** Free margin of the dorsal fin is mainly slightly convex, rarely straight; anal fin most often slightly convex, rarely straight or slightly concave. Origin of anal fin in half the cases at the level of posterior insertion of the dorsal fin, often slightly ahead, rarely slightly behind. Third-fourth type of breast scalation with a predominance of fourth type.

***Coloration.*** Outside the spawning period, males and females are olive-yellow-green in color with very little contrast between horizontal zones and vertical stripes. In general, females have less and males have more saturated coloration. The horizontal stripe along the back of the body is practically not expressed in adults and weakly expressed in juveniles.

***Sexual dimorphism.*** There are significant differences in 7 out of 41 morphometric characters (Table 2). In addition to classical sex characteristics in *Phoxinus* minnows (narrower pectoral fins and less bright colors in females), females have a lower head depth at nape relative to the length of the head and body and greater pectoral – pelvic-fin origin length.

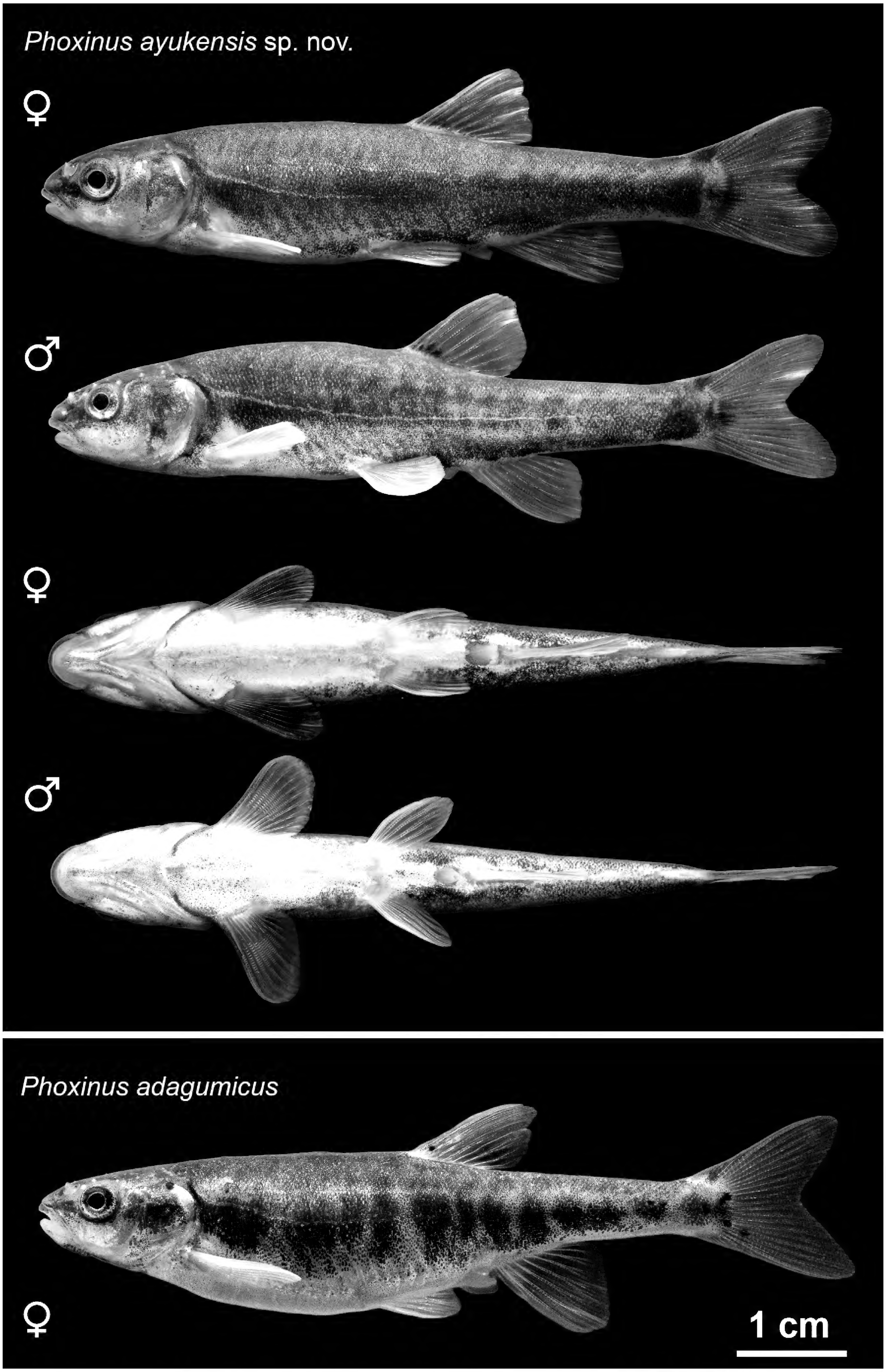


**Figure 3.** Live coloration of female and male of *Phoxinus ayukensis* sp. nov. (paratypes, IBIW_FS_502) from type locality (Ayuk Stream) and *P. adagumicus*, another congeneric species inhabiting the Kuban River basin (female with eggs; 44.4960°N, 38.4330°E; 8 May 2022; IBIW_FS_338) from Zybza River. Side and ventral views are of the same specimens. Photo by Oleg Artaev.

**Table 2.** Morphometrics of *Phoxinus ayukensis* sp. nov. from the type locality (Ayuk Stream) (mean ± SD – bold, and ranges) with level of significance of sex-related differences (primary data see Suppl. material 1). The sign * means the difference between females and males, Mann-Whitney U test: ns ($p > 0.05$), + ($p < 0.05$), ++ ($p < 0.01$).

| Characters | Holotype; (female) | Females, n = 10 | Males, n = 5 | *p** |
|---|---|---|---|---|
| SL | 54.4 | **45.1 ± 6.9**; 37.5–54.5 | **46.1 ± 5.7**; 39.5–54.8 | |
| In percentage of standard length (% SL) | | | | |
| Body depth at dorsal-fin origin | 20.4 | **18.5 ± 1.6**; 17.0–21.8 | **18.9 ± 1.1**; 17.5–20.8 | ns |
| Body width at dorsal-fin origin | 12.7 | **12.8 ± 1.2**; 11.4–15.8 | **11.7 ± 1.2**; 10.6–13.9 | ns |
| Minimum depth of caudal peduncle | 9.8 | **9.0 ± 0.6**; 8.3–10.1 | **9.2 ± 0.7**; 8.3–10.3 | ns |
| Caudal peduncle width | 8.3 | **7.8 ± 0.4**; 7.4–8.4 | **7.6 ± 0.5**; 7.0–8.2 | ns |
| Predorsal length | 58.7 | **57.0 ± 1.1**; 56.1–58.7 | **55.6 ± 1.1**; 54.5–56.9 | ns |
| Postdorsal length | 33.5 | **34.3 ± 0.9**; 33.3–35.7 | **33.4 ± 1.3**; 32.4–35.9 | ns |
| Prepelvic length | 52.6 | **51.0 ± 1.6**; 48.5–53.0 | **50.4 ± 1.8**; 48.5–52.9 | ns |
| Preanal length | 69.7 | **66.9 ± 1.5**; 65.1–69.7 | **64.5 ± 1.4**; 62.9–66.3 | + |
| Pectoral – pelvic-fin origin length | 28.3 | **25.5 ± 1.2**; 24.3–28.3 | **23.5 ± 0.8**; 22.4–24.3 | ++ |
| Pelvic – anal-fin origin length | 19.7 | **17.5 ± 1.2**; 16.1–19.7 | **17.2 ± 1.1**; 15.5–18.9 | ns |
| Caudal peduncle length | 20.2 | **23.0 ± 1.2**; 20.2–24.3 | **24.1 ± 1.2**; 22.5–26.0 | ns |
| Dorsal-fin base length | 12.9 | **11.9 ± 1.0**; 10.2–13.4 | **11.8 ± 0.7**; 10.7–12.3 | ns |
| Dorsal-fin depth | 19.0 | **20.1 ± 1.5**; 17.5–21.7 | **19.5 ± 1.1**; 17.5–20.5 | ns |
| Anal-fin base length | 10.6 | **10.6 ± 0.4**; 9.9–11.1 | **11.0 ± 0.6**; 10.2–11.6 | ns |
| Anal-fin depth | 19.8 | **20.2 ± 1.2**; 18.7–21.8 | **19.4 ± 0.8**; 18.2–20.6 | ns |
| Pectoral-fin length | 17.2 | **18.1 ± 1.8**; 15.1–21.5 | **18.7 ± 1.1**; 17.6–20.7 | ns |
| Pelvic-fin length | 14.1 | **14.8 ± 0.7**; 14.0–16.3 | **15.4 ± 1.0**; 14.5–17.2 | ns |
| Head length | 27.1 | **26.4 ± 1.1**; 24.8–28.5 | **26.5 ± 1.1**; 24.9–27.7 | ns |
| Head depth at nape | 16.6 | **15.9 ± 0.9**; 14.6–17.4 | **17.2 ± 0.9**; 16.2–18.1 | + |
| Maximum head width | 15.3 | **14.3 ± 0.8**; 13.2–15.5 | **14.4 ± 0.9**; 13.6–15.7 | ns |
| Snout length | 8.6 | **7.1–8.6**; 8.0 ± 0.5 | **7.7–9.8**; 8.4 ± 0.8 | ns |
| Eye horizontal diameter | 7.6 | **7.9 ± 0.5**; 7.0–8.7 | **7.6 ± 0.4**; 7.0–8.0 | ns |
| Interorbital width | 9.6 | **9.6 ± 0.3**; 9.1–10.1 | **9.5 ± 0.5**; 8.8–10.1 | ns |
| In percentage of head length (% HL) | | | | |
| Maximum head width | 56.3 | **54.3 ± 1.4**; 52.5–56.3 | **54.5 ± 4.4**; 51.2–63.0 | ns |
| Snout length | 31.6 | **30.5 ± 1.7**; 28.1–32.6 | **31.9 ± 3.3**; 27.8–36.8 | ns |
| Head depth at nape | 61.3 | **60.2 ± 2.3**; 57.0–64.5 | **65.1 ± 4.0**; 61.0–71.9 | + |
| Head depth through eye | 50.3 | **49.2 ± 1.3**; 47.0–51.0 | **51.2 ± 1.9**; 48.8–53.7 | ns |
| Eye horizontal diameter | 27.9 | **29.9 ± 1.7**; 27.5–32.5 | **28.9 ± 1.9**; 25.4–30.9 | ns |
| Postorbital distance | 43.0 | **46.0 ± 2.7**; 42.8–49.8 | **45.3 ± 2.6**; 42.1–50.0 | ns |
| Interorbital width | 35.5 | **36.4 ± 2.0**; 33.8–40.2 | **35.8 ± 1.4**; 33.1–37.0 | ns |
| In percentage of caudal peduncle length | | | | |
| Minimum depth of caudal peduncle | 48.6 | **39.4 ± 4.2**; 35.9–48.6 | **38.1 ± 1.6**; 35.6–39.5 | ns |
| In percentage of body depth | | | | |
| Head length | 133.1 | **142.9 ± 7.4**; 130.9–152.9 | **140.2 ± 11.4**; 120.2–151.6 | ns |
| In percentage of interorbital width | | | | |
| Eye horizontal diameter | 78.6 | **82.4 ± 5.0**; 74.8–90.0 | **80.8 ± 6.6**; 69.7–89.7 | ns |
| Ratios: | | | | |
| Interorbital width/eye horizontal diameter | 1.3 | **1.2 ± 0.1**; 1.1–1.3 | **1.2 ± 0.1**; 1.1–1.4 | ns |
| Snout length/eye horizontal diameter | 1.1 | **1.0 ± 0.1**; 0.9–1.2 | **1.1 ± 0.1**; 1.0–1.2 | ns |
| Head depth at nape/eye horizontal diameter | 2.2 | **2.0 ± 0.1**; 1.8–2.2 | **2.3 ± 0.2**; 2.0–2.5 | + |
| Head length/caudal peduncle depth | 2.8 | **2.9 ± 0.1**; 2.8–3.1 | **2.9 ± 0.3**; 2.4–3.2 | ns |
| Length of caudal peduncle/caudal peduncle depth | 2.1 | **2.6 ± 0.2**; 2.1–2.8 | **2.6 ± 0.1**; 2.5–2.8 | ns |
| Pectoral fin length/pectoral – pelvic-fin origin distance | 0.6 | **0.7 ± 0.1**; 0.6–0.8 | **0.8 ± 0.1**; 0.7–0.9 | + |
| Predorsal length/head length | 2.2 | **2.2 ± 0.1**; 2.0–2.3 | **2.1 ± 0.1**; 2.0–2.2 | ns |
| Body width at dorsal-fin origin/Caudal peduncle depth | 1.3 | **1.4 ± 0.1**; 1.3-1.6 | **1.3 ± 0.1**; 1.2-1.5 | + |

**Etymology.** The new species is named after the Ayuk (= Burlachenkova Shchel, Peshchernaya Shchel, Semenovskaya Shchel) Stream – the only known habitat area. Name of the Ayuk Stream apparently comes from the Adyghe language word "ayuko", which means "unfriendly valley". Ayukensis – an adjective.

**Taxonomic remarks.** Taxonomic affiliation of *Phoxinus* minnows from the Ayuk Stream, along with minnows from the lower Kuban basin, which were recently described as a separate species (Artaev et al. 2024a), was questionable and was cautiously attributed to both *P. phoxinus* (Sukhanova and Troitsky 1949) and *Phoxinus phoxinus kubanicum* [sic] (Otrishko and Emtyl 2013a) and *Phoxinus kubanicus* [sic] (Otrishko and Emtyl 2013b). The latter two cannot be considered as available names (Bogutskaya et al. 2023, Artaev et al. 2024a). Also, based on a discriminant analysis of the external morphological characteristics of minnows from the Ayuk Stream and Durso River (the latter is inhabited by *P. colchicus*), it was proposed that minnows from the Ayuk Stream "attributed

**Table 3.** Meristic characters of *Phoxinus ayukensis* sp. nov., including the type material from the type locality Ayuk Stream (primary data; see Suppl. material 1).

| Characters | Mean ± SD; range | n |
|---|---|---|
| Total number of scales in lateral series (sql) | **97.7 ± 5.0**; 87–105 | 15 |
| Total number of lateral-line (pored) scales (llt) | **30.5 ± 12.2**; 9–54 | 14 |
| Number of pored scales in first complete (non-interrupted) section of lateral line (llcs) | **18.4 ± 10.3**; 5–36 | 14 |
| Relative number of total lateral-line scales, quotient llt:sql (lltr) | **0.31 ± 0.1**; 0.09–0.56 | 14 |
| Mean number of scale rows on left and right breast patches (BrPScale) | **6.5 ± 0.9**; 5–7.5 | 15 |
| Number of circumpeduncular scales (cps) | **45.1 ± 2.0**; 41–49 | 15 |
| Scales above lateral line (between lateral line and base of first unbranched ray in D) (all) | **21.1 ± 1.7**; 19–23 | 11 |
| Scales below lateral line (between lateral line and base of first unbranched ray in A) (bll) | **15.3 ± 1.6**; 13–18 | 11 |
| Scales between bases of dorsal and anal fins (bda) | **36.7 ± 2.9**; 32–41 | 15 |
| Pattern of scalation on the breast and anterior belly (cstyp) | **4**; 3–4 | 15 |
| Total number of left pectoral-fin rays (P) | **15.4 ± 0.6**; 14–16 | 15 |
| Total number of left pelvic-fin rays (V) | **7.64 ± 0.5**; 7–8 | 25 |
| Number of branched dorsal-fin rays (with 1/2) (D) | **7.0 ± 0.0**; 7–7 | 15 |
| Number of branched anal-fin rays (with 1/2) (A) | **7.0 ± 0.0**; 7–7 | 15 |
| Total number of rays in caudal fin (C) | **19.0 ± 0.4**; 18–20 | 15 |
| Total number of vertebrae (tv) | **40.1 ± 0.6**; 39–41 | 26 |
| Number of abdominal vertebrae (abdv) | **22.7 ± 0.8**; 21–24 | 26 |
| Number of caudal vertebrae (caudv) | **17.4 ± 0.8**; 16–19 | 26 |
| Number of predorsal abdominal vertebrae (preDv) | **14.5 ± 0.5**; 14–15 | 26 |
| Number of anal-fin pterygiophores in front of the first caudal vertebrae (preAp) | **5.7 ± 0.9**; 4–7 | 25 |
| Difference between numbers of abdominal and caudal vertebrae (dac) | **5.2 ± 1.5**; 2–8 | 26 |
| Gill rakers in first left arch | **7.9 ± 0.8**; 7–9 | 22 |

to the subspecies of Colchis minnow" (Emtyl et al. 1994). However, these assumptions were based on insufficient data and cannot be considered taxonomically valid.

**Type locality.** Ayuk (= Burlachenkova Shchel, Peshchernaya Shchel, Semenovskaya Shchel) Stream (44.4756°N, 39.0196°E) upstream of Fanagoriyskoe Vil., Krasnodar Krai, Russia (Fig. 4). A tributary of the Chepsi River → Psekups River → Kuban River (Krasnodar Reservoir) → Sea of Azov.

**Distribution and habitats.** New species has an extremely narrow range and is known from the Ayuk Stream only in the area of the Ayuk waterfalls and above them (Fig. 4). The total length of the river is about 12 km, the upper stream of which (5–7 km) is inhabited by the core population. Some individuals occasionally migrate downstream to the confluence with the Chepsi River (about 5.5 km) (Fig. 5). In the summer, the water level is low, surface runoff stops, and water pools remain in the deep parts of the riverbed, which are apparently supplied by interstitial waters (Fig. 4A). Such water pools are inhabited by *P. ayukensis* sp. nov. only.

**Morphological comparisons.** Cluster analysis of the mean values of 41 morphometric characters for five geographically close species demonstrates that *Phoxinus ayukensis* sp. nov. is the most distinct, not only from the closely related and neighboring *P. adagumicus* but also from other Crimean and Caucasian species, and is most similar to the northwardly distributed *P. isetensis* (Fig. 6A). The discriminant function analysis of eight external meristic characters effectively differentiates the new species from the other four species under comparison (Fig. 6B): DFA statistics values are as follows: Wilks' $\lambda = 0.03094$, approx. F (56.103) = 16.74, $p < 0.0000$. The PCA analysis of eight meristic traits has also demonstrated clear separation of the new species (Fig. 7). Notably, the characters contributing to the separation of new species are related to the body squamation: cps, bda, and sql.

The new species differs from the other European *Phoxinus* species by a significantly increased number of scales in the lateral series (Table 4). The increased squamation of the new species is also characteristic of the number of scales between the bases of the dorsal and anal fins. Another feature that distinguishes the new species from the six other studied species is the high frequency of reduction of the total number of rays to 7 in the pelvic fins observed in each third specimen (Fig. 8D).

Compared to *Phoxinus abanticus* from the Lake Abant basin in Türkiye (Turan et al. 2023), *P. ayukensis* sp. nov. has a lower body – body depth 17.0–21.8% SL (vs. 22–25%); 87–105 total number of scales in lateral series (vs. 60–69); 19–23 scales above lateral line (vs. 11–14); 13–18 scales below lateral line (vs. 7–10); scales on breast patches in males (vs. no scales in males).

Compared to *Phoxinus adagumicus* from another part of the Kuban system, Adagum River basin, Russia (Artaev et al. 2024a), *P. ayukensis* sp. nov. has larger eye horizontal diameter 27.5–32.5%, mean 29.9% HL (vs. 22.9–28.5%, mean 25.9%) in females and 24.5–30.9%, mean 28.9% HL (vs. 21.8–25.9%, mean 24.5%) in males; 87–105, mean 97.7 total number of scales in lateral series (vs. 74–94, mean 84.5); 32–41, mean 36.7 scales between bases of dorsal and anal fins (vs. 26–36, mean 31.2); 13–18, mean 15.3 scales below lateral line (vs. 8–14, mean 11.8); 4–7, mean 5.7 number of anal-fin pterygiophores in front of the first caudal vertebrae (vs. 3–7, mean 4.4); 7–8, mean 7.6 rays in pelvic fins (vs. 7–9, mean 8.0); absence of single-row pharyngeal teeth (vs. dominance) and the predominance of the classical dental formula for the genus - 2.5–4.2 (vs. predominance 5-4) (Suppl. materials 2, 3).


**Figure 4.** Habitat of *Phoxinus ayukensis* sp. nov.: **A.** Puddle in the bed of Ayuk Stream, sampling place of the type material (44.4756°N, 39.0194°E, 19 July 2024, photograph by Oleg Artaev); **B.** One of a series of waterfalls that restrict the distribution of *P. ayukensis* sp. nov. (44.4749°N, 39.0209°E, 9 March 2025, photograph by Andrey Ostapenko).

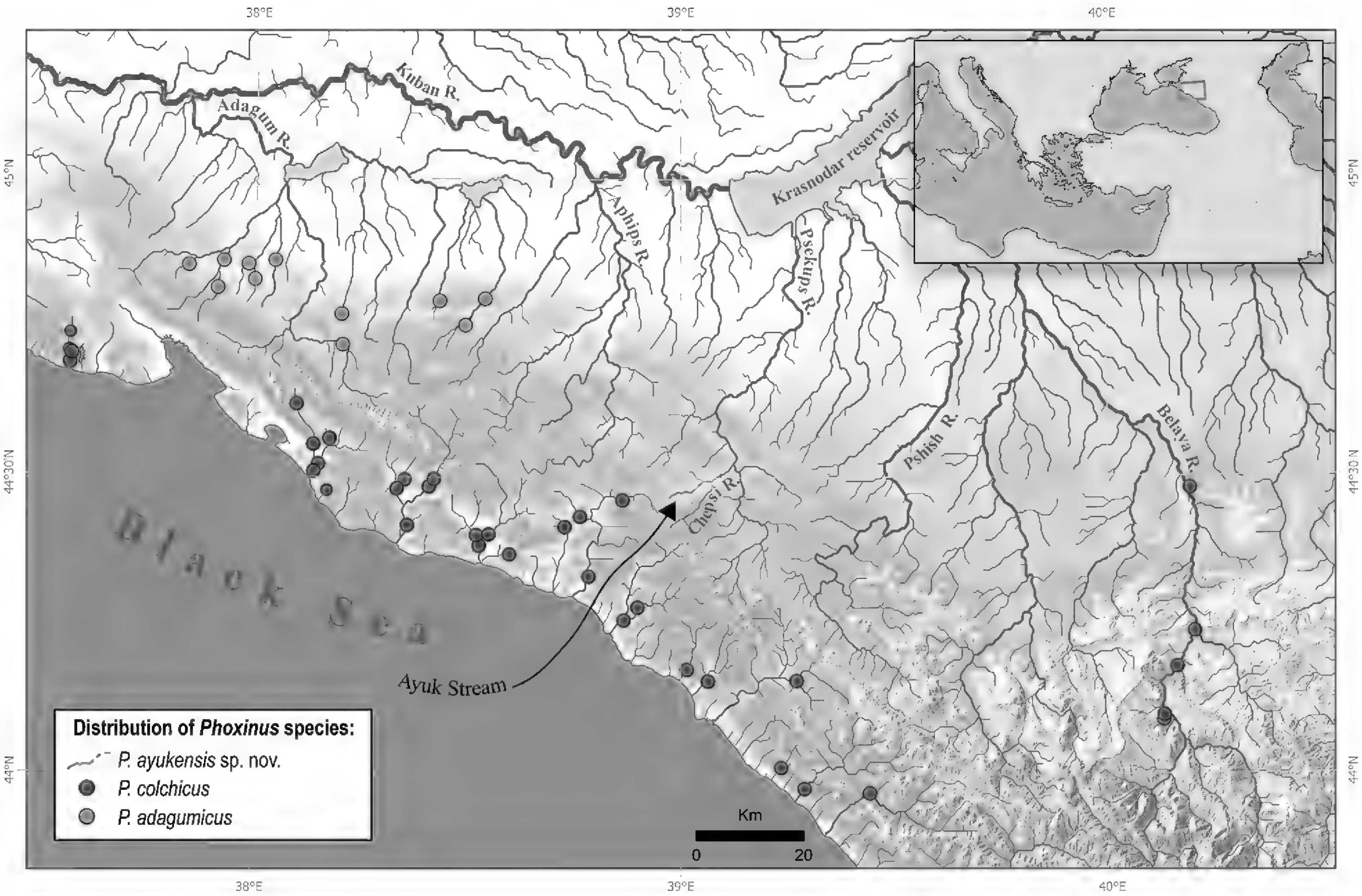


**Figure 5.** Map of *Phoxinus* species distribution in the Northwestern Caucasus (based on own data).

Compared to *Phoxinus bigerri* from the Adour and the Leyre drainages in France (Denys et al. 2020), *P. ayukensis* sp. nov. has 87–105 total number of scales in lateral series (vs. 68–87). Compared to *P. bigerri* from Estuary of Bilbao in Spain (Palandačić et al. 2024) has 87–105, mean 97.7 total number of scales in lateral series (vs. ca. 66–85, mean 76.6); 9–54, mean 30.5 numbers of lateral-line scales (pored) (vs. ca. 31–80, mean 66.0); $3^{rd}$–$4^{th}$ types of scalation pattern of the breast and anterior belly with predominance of $4^{th}$ type (vs. $3^{rd}$–$7^{th}$, $9^{th}$, $11^{th}$, and $12^{th}$ types with predominance of $3^{rd}$ type) (Suppl. material 3).

Compared to *Phoxinus chrysoprasius* from the rivers of the Crimean Peninsula (Bogutskaya et al. 2023; Artaev et al. 2024a), *P. ayukensis* sp. nov. has larger eye horizontal diameter 27.5–32.5%, mean 29.9% HL (vs. 18.5–26.0%, mean 23.5%) in females; 87–105, mean 97.7 total number of scales in lateral series (vs. 78–104, mean 89.9); 7–8, mean 7.6 rays in pelvic fins (vs. 7–9, mean 8.0); 14–16, mean 15.4 rays in pectoral fins (vs. 13–19, mean 17.0); 9–54, mean 30.5 lateral-line (pored) scales (vs. 46– ca. 100, mean 72.8) (Suppl. materials 2, 3).

Compared to *Phoxinus colchicus* from the Kuban basin and rivers of the Black Sea coast of the Caucasus (Artaev et al. 2024a), *P. ayukensis* sp. nov. has larger head length 130.9–152.9%, mean 142.9% to body depth (vs. 97.4–129.8%, mean 111.6%) in females and 120.2–151.6%, mean 140.2% (vs. 106.6–139.1%, mean 120.9%) in males; 87–105, mean 97.7 total number of scales in lateral series (vs. 76–96, mean 82.9); 5–7, mean 6.4 number of scale rows on left and right breast patches (vs. 5–10, mean 8.0); 41–49, mean 45.1 circumpeduncular scales (vs. 36–48, mean 40.9); 32–41, mean 36.7 scales between bases of dorsal and anal fins (vs. 30–37, mean 33.0); $3^{rd}$–$4^{th}$ types of scalation pattern of the breast and anterior belly with predominance of $4^{th}$ type (vs. $3^{rd}$–$10^{th}$, $13^{th}$, and $14^{th}$ types with predominance of $6^{th}$ type); 7–8, mean 7.6 rays in pelvic fins (vs. 8–9, mean 8.1) (Suppl. materials 2, 3).

Compared to *Phoxinus csikii* from the Danube River basin, Montenegro, and Bulgaria (Bogutskaya et al. 2019, 2023), *P. ayukensis* sp. nov. has 87–105, mean 97.7 total number of scales in lateral series (vs. ca. 71–100, mean 84.8); 9–54, mean 30.5 lateral-line (pored) scales (vs. ca. 11–80, mean 52.9); $3^{rd}$–$4^{th}$ types of scalation pattern of the breast and anterior belly with predominance of $4^{th}$ type (vs. $3^{rd}$–$9^{th}$ and $11^{th}$ types with predominance of $7^{th}$ type) (Suppl. material 3).

Compared to *Phoxinus dragarum* from the Garonne drainage in France (Denys et al. 2020), *P. ayukensis* sp. nov. has 87–105 total number of scales in lateral series (vs. 70–97); 9–54 lateral-line (pored) scales (vs. 50–74).

Compared to *Phoxinus fayollarum* from the Loire drainage in France (Denys et al. 2020), *P. ayukensis* sp. nov. has 87–105 total number of scales in lateral series (vs. 72–91); 9–54 lateral-line (pored) scales (vs. 56–84).

Compared to *Phoxinus isetensis* from basins of Baltic, Barents, Caspian Seas and Kara Sea basin (Artaev et al. 2024b), *P. ayukensis* sp. nov. has 87–105, mean 97.7 total number of scales in lateral series (vs. 71–103, mean

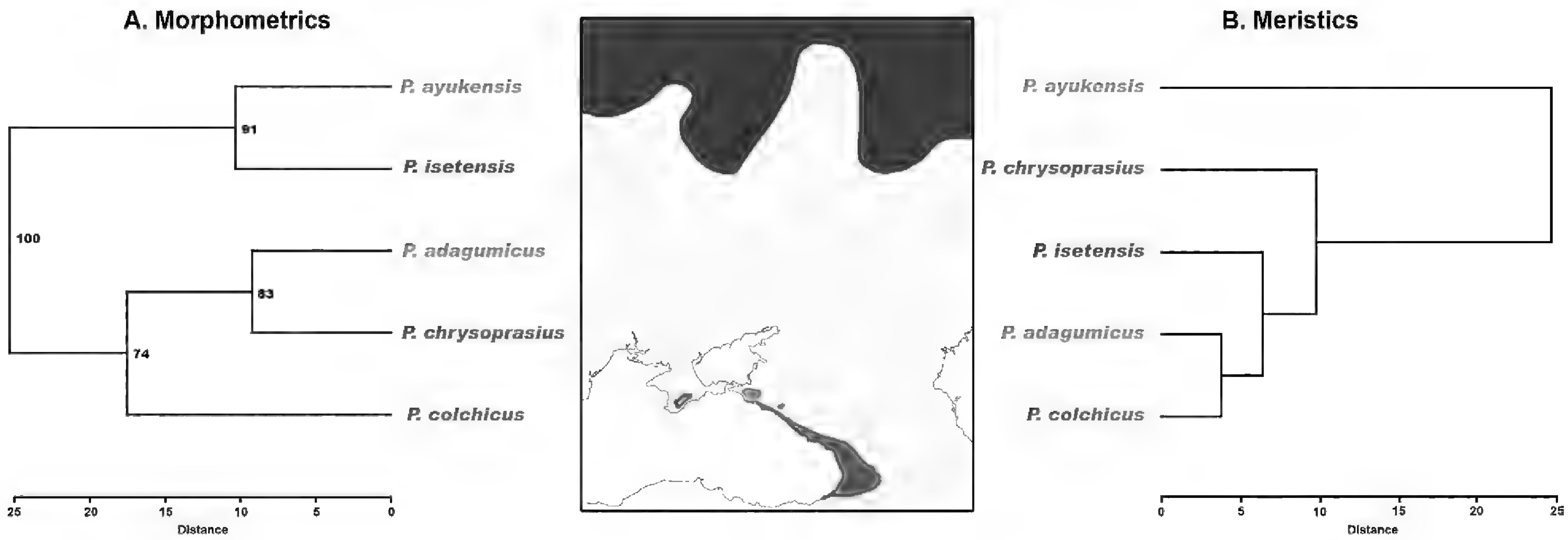


**Figure 6.** Dendrograms (UPGMA): **A.** Average values of 41 morphometric characters for each species with bootstrap support on each node (Euclidean distance); **B.** Eight external meristic characters based on squared Mahalanobis distances defined by discriminant functions in DFA. In both cases, the difference was statistically significant between all species compared ($p < 0.005$). A schematic map of species ranges in the center, with colors corresponding to the species names.

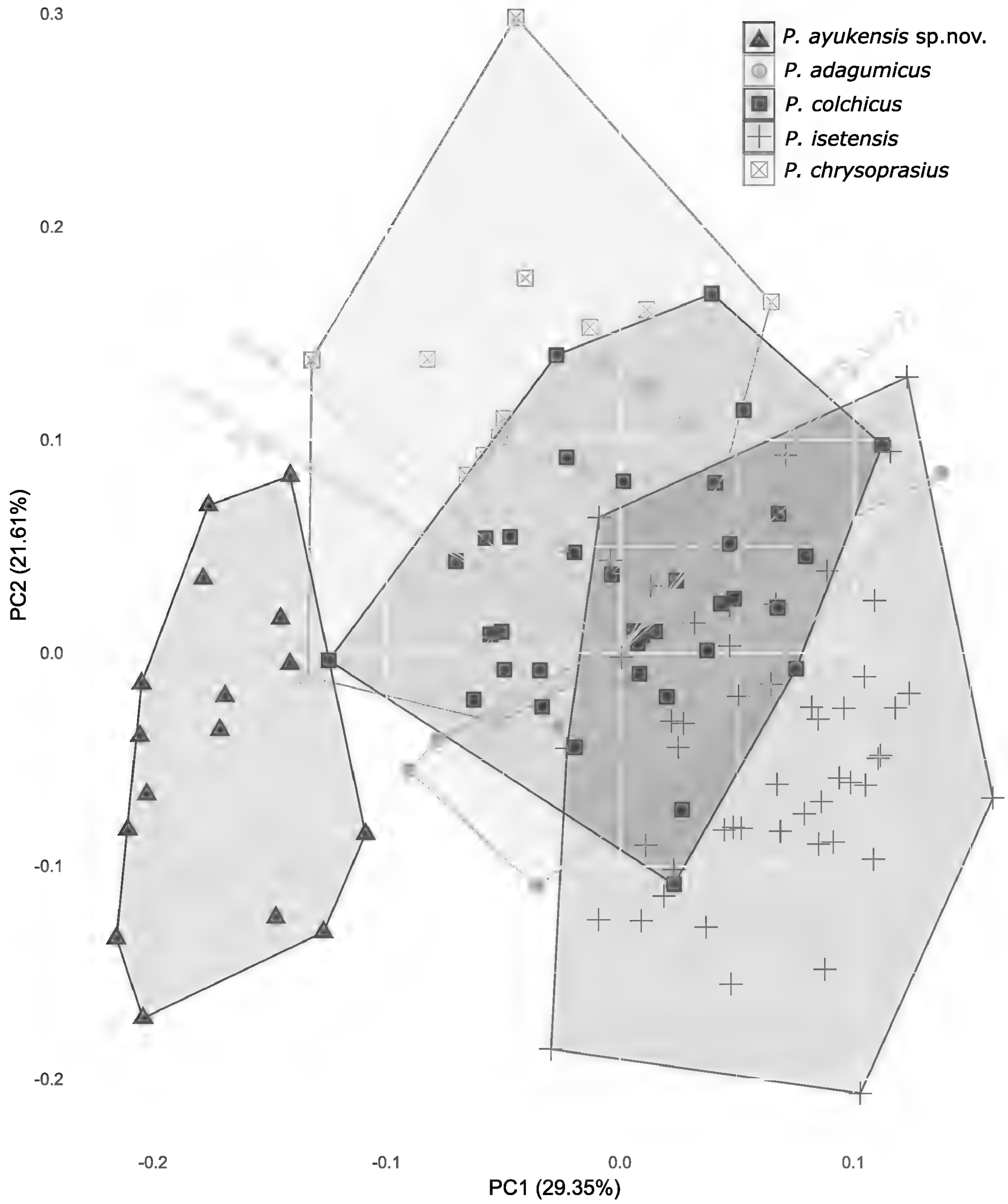


**Figure 7.** PCA of *Phoxinus* spp. based on eight meristic characters and loading plot showing how strongly each character influences principal components.

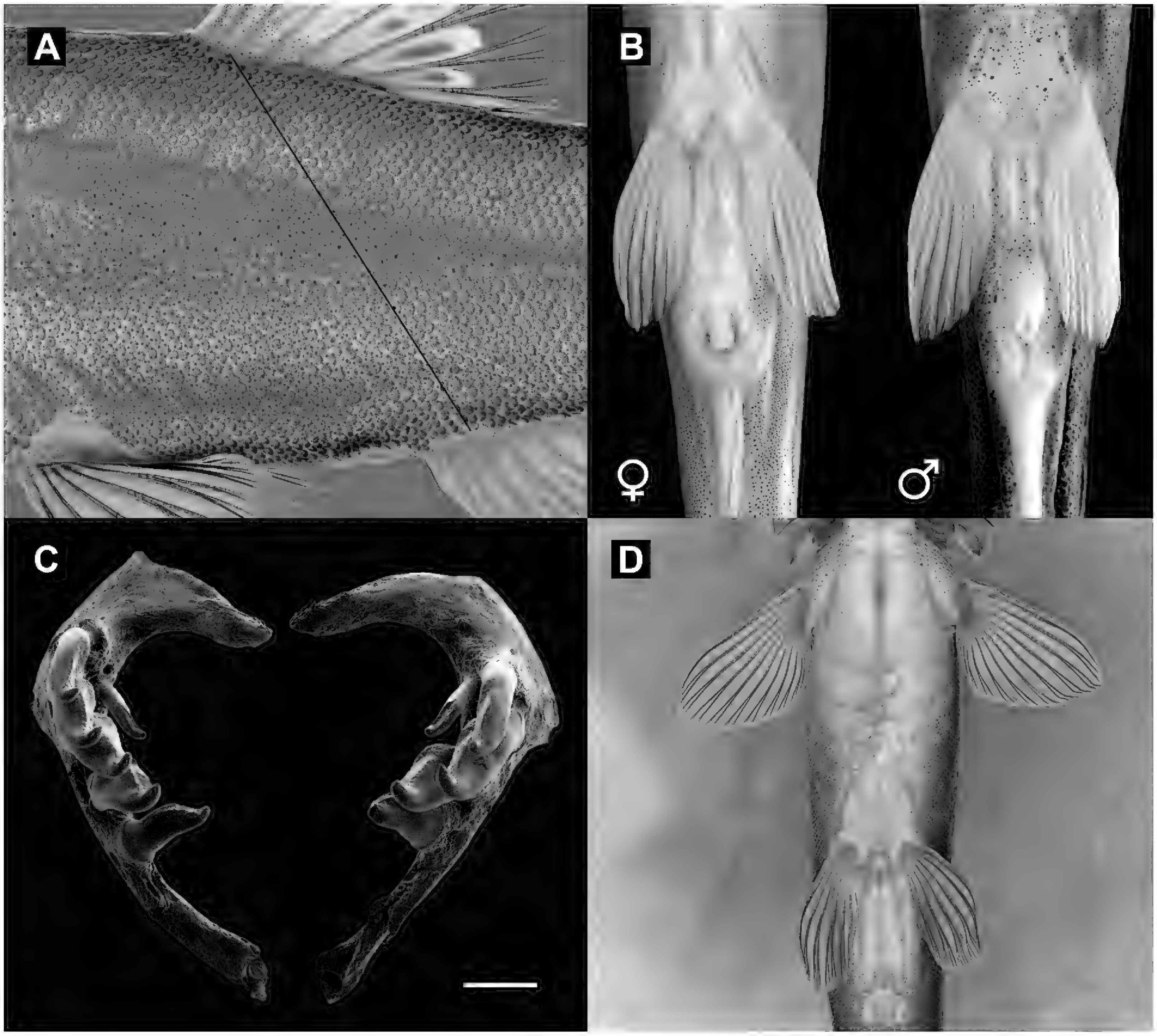


**Figure 8.** Some morphological features of *Phoxinus ayukensis* sp. nov. **A.** Example of squamation of middle part of body of an alizarin-stained specimen (colors changed): a diagonal line marks the direction of scale counting between first unbranched rays of dorsal and anal fins (bda), above (all) and below (bll) lateral line (bda = 36, all = 21, and bll = 14 scales); **B.** Ventral view of pelvic fins, genital and anal openings in females and males; **C.** Dentition of pharyngeal bones: double-row formula 1.5–4.1 (observed in 10% of specimens); **D.** Ventral view of an alizarin-stained specimen showing 3rd-type scalation on the breast and belly and reduction of ray numbers to seven in both pelvic fins. Scale bar: 0.5 mm (**C**).

**Table 4.** Total number of scales in the lateral series (sql) of *Phoxinus ayukensis* sp. nov. and other *Phoxinus* species from Europe (detailed data see Suppl. material 3).

| Number of scales / species or clades | 71–75 | 76–80 | 81–85 | 86–90 | 91–95 | 96–100 | 101–105 | Mean | n | Reference |
|---|---|---|---|---|---|---|---|---|---|---|
| *P. ayukensis* sp. nov. | – | – | – | 1 | 4 | 5 | 5 | 97.7 | 15 | This study |
| *P. adagumicus* | 1 | 5 | 12 | 9 | 2 | – | – | 84.5 | 29 | Artaev et al. 2024b |
| *P. colchicus* | | 8 | 13 | 18 | 5 | 2 | – | 82.9 | 46 | Bogutskaya et al. 2023; Artaev et al. 2024b |
| *P. chrysoprasius* | – | 1 | 9 | 25 | 11 | 6 | 3 | 89.9 | 55 | Bogutskaya et al. 2023; Artaev et al. 2024b |
| *P. csikii*, Clade 5 | 1 | 29 | 59 | 28 | 6 | 1 | – | 84.8 | 124 | Bogutskaya et al. 2019 |
| *P. isetensis* | 2 | 18 | 53 | 49 | 16 | 8 | 3 | 85.7 | 149 | Artaev et al. 2024a |
| *P. strandjae*, Clade 14 | – | 3 | 24 | 76 | 28 | 5 | – | 88.3 | 136 | Bogutskaya et al. 2023 |
| *P. krkae*, Clade 6 | 14 | 33 | 18 | – | – | – | – | 78.5 | 65 | Bogutskaya et al. 2019 |
| *P. lumaireul*, Clade 1a | 25 | 69 | 67 | 10 | – | – | – | 80.3 | 171 | Bogutskaya et al. 2019 |
| *P. lumaireul*, Clade 1b | 14 | 52 | 27 | 9 | 1 | – | – | 81.4 | 103 | Bogutskaya et al. 2019 |
| *P. marsilii*, Clade 9 | 1 | 4 | 12 | 12 | 4 | – | – | 84.0 | 33 | Bogutskaya et al. 2023 |
| *P. septimaniae*, Clade 12 | – | – | 5 | 6 | – | – | – | 85.6 | 11 | Bogutskaya et al. 2019 |
| *P.* sp., Clade 2 | 1 | 4 | 10 | 1 | – | – | – | 82.7 | 16 | Bogutskaya et al. 2019 |

85.7); 9–54, mean 30.5 lateral-line (pored) scales (vs. 23–66, mean 50.8); 41–49, mean 45.1 circumpeduncular scales (vs. 33–39, mean 35.2); 19–23, mean 21.1 scales above lateral line (vs. 14–18, mean 15.4); 13–18, mean 15.3 scales below lateral line (vs. 9–14, mean 11.4); 32–41, mean 36.7 scales between bases of dorsal and anal fins (vs. 22–35, mean 27.7); 7–8, mean 7.6 rays in pelvic fins (vs. 7–9, mean 8.0); 39–41, mean 40.0 total number of vertebrae (vs. 39–42, mean 41.0); 16–19, mean 17.3 caudal vertebrae (vs. 16–21, mean 18.9); difference between numbers of abdominal and caudal vertebrae is 2–8, mean 5.3 (vs. 0–7, mean 3.2) (Suppl. material 3).

Compared to *Phoxinus karsticus* from Lake Skadar (Palandačić et al. 2024), *P. ayukensis* sp. nov. has 97.7 mean number of total number of scales in lateral series (ca. 76–90, mean vs. 80.4); 3rd–4th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 3rd–7th, 9th types with predominance of 3rd type).

Compared to *Phoxinus krkae* from the Krka River, Croatia (Bogutskaya et al. 2019), *P. ayukensis* sp. nov. has 87–105, mean 97.7 total number of scales in lateral series (vs. 72–84, mean 78.5); 3rd–4th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 3rd–7th types with predominance of 6th type); 39–41, mean 40.0 total number of vertebrae (vs. 37–40, mean 38.4); 21–24, mean 22.8 abdominal vertebrae (vs. 21–22, mean 21.6) (Suppl. material 3).

Compared to *Phoxinus lumaireul* clades 1a and 1b from the rivers in the Adriatic and Black Sea basins in Italy, Slovenia, and Croatia (Bogutskaya et al. 2019), *P. ayukensis* sp. nov. has 87–105, mean 97.7 total number of scales in lateral series (vs. ca. 71–105, mean 82.3); 3rd–4th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 2nd –7th types with predominance of 3rd type) (Suppl. material 3).

Compared to *Phoxinus marsilii* from the Danube River basin, Austria and Croatia (Bogutskaya et al. 2019, 2023), *P. ayukensis* sp. nov. has 87–105, mean 97.7 total number of scales in lateral series (vs. ca. 71–95, mean 84.0); 9–54, mean 30.5 lateral-line (pored) scales (vs. ca. 11–90, mean 65.4); 3rd–4th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 3rd–8th types with predominance of 6th type); 21–24, mean 22.8 abdominal vertebrae (vs. 20–23, mean 21.8); difference between numbers of abdominal and caudal vertebrae is 2–8, mean 5.3 (vs. 1–6, mean 3.0) (Suppl. material 3).

Compared to *Phoxinus phoxinus* from France (Denys et al. 2020), *P. ayukensis* sp. nov. has an almost non-overlapping caudal peduncle depth 2.1–2.8 times its length (vs. 2.8–3.9).

Compared to *Phoxinus radeki* from the Ergene River (Aegean Sea basin) in Türkiye (Bayçelebi et al. 2024), *P. ayukensis* sp. nov. has 87–105 total number of scales in lateral series (vs. 75–96); 19–23 scales above lateral line (vs. 9–15); 13–18 scales below lateral line (vs. 6–9); 14–16 rays in pectoral fins (vs. 16–18).

Compared to *Phoxinus septimaniae* from the Herault River, France (Bogutskaya et al. 2019), *P. ayukensis* sp. nov. has 87–105, mean 97.7 total number of scales in lateral series (vs. ca. 81–90, mean 85.6 or 75–94 (Denys et al. 2020)); 3rd–4th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 12th–14th types with predominance of 14th type); 21–24, mean 22.8 abdominal vertebrae (vs. 21–23, mean 21.8) (Suppl. material 3).

Compared to *Phoxinus strandjae* from the rivers of the Black Sea basin, Bulgaria, and the rivers of the Marmara Sea, Türkiye (Bogutskaya et al. 2019, 2023), *P. ayukensis* sp. nov. has 87–105, mean 97.7 total number of scales in lateral series (vs. ca. 76–100, mean 88.3); 3rd–4th types of scalation pattern of the breast and anterior belly with predominance of 4th type (vs. 3rd–12th types with predominance of 6th, 7th, 9th, and 11th types) (Suppl. material 3).

# Discussion

This study has discovered a new species of minnow in Caucasus fresh waters and clarified its morphology, phylogenetic position, genetic diversity, and distribution. *Phoxinus ayukensis* sp. nov. has an exceptionally narrow range in the Kuban River basin—the richest in endemic species compared to other rivers in European Russia (Abell et al. 2008). The new species is moderately divergent from the close relative species *P. adagumicus* in genetics, but it is significantly different from the latter and the other known species by morphology.

Disjunctive distribution of closely related *Phoxinus* species in the Kuban basin (Fig. 5) suggests that range of mitochondrial clade has been wider in the past but was shrunken, likely due to climatic oscillations. In particular, we assume that the common ancestor of the *P. adagumicus* and *P. ayukensis* sp. nov. had a continuous range, which covered the distribution of both species, but the Pleistocene glaciations might have fragmented the historical range. The impact of Pleistocene glaciations in the Caucasus was most notable in the northwestern Caucasus, including the Kuban riverine system (Boriskovskii 1984; Gobejishvili et al. 2011; Doronicheva et al. 2023). Therefore, the last glaciations might shrink the historical range of *Phoxinus* minnows in the Caucasus and result in the disjunct range of surviving populations that could evolve as independent species. The narrow-ranged *Phoxinus ayukensis* sp. nov. represents a relic population/lineage that could adapt to the relatively stable hydrothermal conditions of the Ayuk Stream (in summer the water is colder than in surrounding rivers) supplied by groundwater discharges in the karst region near the Bolshaya Fanagoriyskaya Cave. The distinct morphology of this species—first of all, the highest number of scales—is a good sign of the environmental impact on phenotype—low temperature at the stage of scale morphogenesis, in particular (reviewed in Levin 2011). The habitat of species in the upper reaches of the Ayuk Stream has three peculiar features: i) it is isolated from the lower part of the stream by Ayuk waterfalls (see Fig. 4B), ii) it is nearby one of the largest clastokarst caves in the Caucasus—Bolshaya Fanagoriyskaya (= Psekupskaya) Cave

(Gergedava 1990; Ostapenko 1994, 1998; Ostapenko and Kritskaya 2019), and iii) it has a stable cold temperature regime of +6–10 °C (Ostapenko 1998). During the winter and spring, the Ayuk Stream is primarily fed by precipitation, while during the dry season, the flow is sustained by karst springs and outlet cave waters. One of the main sources feeding the Ayuk Stream originates from the Bolshaya Fanagoriyskaya Cave (44.4692°N, 38.9778°E), which was formed by an ancient subterranean stream. The cave's subterranean stream currently has a flow rate of 1–2 L/s, increasing to 20–50 L/s during floods with a stable water temperature of +8.1–8.2 °C (Ostapenko and Kritskaya 2019). However, the majority of the dry-season flow comes from the smaller karst springs, of which there are about ten in the Ayuk Stream valley, with three contributing the most significantly (44.4733°N, 38.9857°E; 44.48125°N, 38.9802°E; and 44.4643°N, 39.0078°E) (A. Ostapenko, pers. comm.). The cave and karst spring outlets could have helped maintain favorable conditions and promoted the survival of species during climatic oscillations, while the waterfalls could isolate the population. New species rarely occur below waterfalls. Only one individual was caught at the confluence with the Chepsi River (44.4987°N, 39.0723°E) in spite of intensive sampling. The fauna of the Ayuk Stream between the Ayuk waterfalls and the confluence with the Chepsi River is relatively rich and represented by *Alburnoides kubanicus* Bănărescu, 1964, *Chondrostoma kubanicum* Berg, 1914, *Petroleuciscus aphipsi* (Aleksandrov, 1927), and *Gobio* sp. Meanwhile, only *P. ayukensis* sp. nov. was recorded above the waterfalls (Sukhanova and Troitskiy 1949; our data). The question of why the species is not distributed out of its modern range remains not understood. One may suggest that *P. ayukensis* sp. nov. is 'too' adapted to cold waters because of the rather stable low temperature in the Ayuk Stream and has lost the possibility to survive in warmer waters. It is rather possible given the small size of the isolated population and the high role of genetic drift, inbreeding, and the weakening of natural selection in such populations, as well as the founder effect (Nei et al. 1975; Frankham 2005).

Remarkably, the phenotype of *Phoxinus ayukensis* sp. nov. is the most distant from the other Caucasian species, but it is closer to the northward-distributed Northern minnow *P. isetensis*. Two non-competing hypotheses could explain this. The first hypothesis suggests adaptation to cold waters (environmental hypothesis). The rapid change in scale numbers and other phenotypic traits in fishes is rather possible due to the heterochronies resulting in higher scale numbers caused by the retardation of early development (Smirnov et al. 2006; Levin and Levina 2014). Taking into account the isolation of the small population of *P. ayukensis* sp. nov. that might undergo restricted gene flow along with a high rate of mutation fixation, its rapid adaptation to a cold-water environment is a very plausible explanation. Similarly, small-sized populations of Crimean minnow *P. chrysoprasius*, which inhabit cold waters in a zone of powerful karst water outlets, are characterized by an increased number of scales in the series of lateral lines (Suppl. material 3). The second hypothesis suggests that the morphology of the *P. ayukensis* sp. nov. might be a result of ancient hybridization with species bearing similar phenotypes during ancient secondary contact of the *Phoxinus* species (past hybridization hypothesis). However, this hypothesis is currently less evident and needs to be tested further using the genomic methods.

Another species of *Phoxinus* minnows with a very limited range, *P. krkae*, lives in the Balkans in the upper reaches of the Krka River in Croatia (Bogutskaya et al. 2019). Remarkably, its range is also located nearby, at a distance of 7–20 km from the spring on the Krka River in the town of Knin (Bogutskaya et al. 2019). Both narrow-ranged endemic *Phoxinus* minnows have significant morphological differences with extreme values for some characters within the genus. In particular, *P. krkae* has minimum values of total and caudal vertebrae, while *P. ayukensis* sp. nov. has maximum values of number of scales in the lateral series and number of scales between bases of dorsal and anal fins, and also a reduced number of rays in pelvic fins (Suppl. material 3). Another species of minnow with a very narrow range, *P. abanticus*, also has features that strongly distinguish it from other *Phoxinus* species—absence of the scales on the breast in males (Turan et al. 2023). These morphological 'abnormalities' in the small isolated populations may be a consequence of genetic processes undergoing in the small isolated populations—e.g., genetic drift, restricted gene flow, etc.—likely combined with a population bottleneck or founder effect (Kimura 1968, Grant and Grant 2002). All this could play a significant role in the emergence of distinct phenotypic traits observed in these species.

Although *Phoxinus ayukensis* sp. nov. is relatively abundant, its very limited range, stenothermic ecology, dependence on the hydrological regime of a single small stream, and low genetic diversity suggest that this species might be threatened. In our opinion, the Ayuk minnow is in need of protection through listing in the Red Books, the IUCN Red List, and other measures to prevent population decline.

# Acknowledgments

The authors are very grateful to Andrey Ostapenko (Krasnodar, Russia) for valuable consultations on the karst spring system and hydrological regime and also for the photograph of the waterfall. The study was supported partially by the Russian Science Foundation (grant 23-14-00128).

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# Supplementary material 1

## Primary morphological data from type locality

Authors: Oleg N. Artaev, Ilya S. Turbanov, Aleksey A. Bolotovskiy, Alexander A. Gandlin, Boris A. Levin
Data type: xlsx
Explanation note: Primary morphological data of *Phoxinus ayukensis* sp. nov. from type locality (Ayuk Stream).

Link: https://doi.org/10.3897/zse.101.153548.suppl1

# Supplementary material 2

## Comparsion of morphometrics

Authors: Oleg N. Artaev, Ilya S. Turbanov, Aleksey A. Bolotovskiy, Alexander A. Gandlin, Boris A. Levin
Data type: xlsx
Explanation note: Morphometrics of *Phoxinus ayukensis* sp. nov., *P. isetensis*, *P. adagumicus*, *P. chrysoprasius*, *P. colchicus* and its comparison.

Link: https://doi.org/10.3897/zse.101.153548.suppl2

# Supplementary material 3

## Meristic and qualitative characters

Authors: Oleg N. Artaev, Ilya S. Turbanov, Aleksey A. Bolotovskiy, Alexander A. Gandlin, Boris A. Levin
Data type: xlsx
Explanation note: Meristic and qualitative characters of *Phoxinus ayukensis* sp. nov. and other *Phoxinus* species published in the literature.

Link: https://doi.org/10.3897/zse.101.153548.suppl3

# Supplementary material 4

## Material for genetic studies

Authors: Oleg N. Artaev, Ilya S. Turbanov, Aleksey A. Bolotovskiy, Alexander A. Gandlin, Boris A. Levin
Data type: xlsx

Link: https://doi.org/10.3897/zse.101.153548.suppl4

# Supplementary material 5

## Best partition schemes

Authors: Oleg N. Artaev, Ilya S. Turbanov, Aleksey A. Bolotovskiy, Alexander A. Gandlin, Boris A. Levin
Data type: docx
Explanation note: The best partition schemes generated by ModelFinder v.2.2.0 (ML) and PartitionFinder v.2.1.1 (BI).

Link: https://doi.org/10.3897/zse.101.153548.suppl5

# Supplementary material 6

## Additional and comparative materials

Authors: Oleg N. Artaev, Ilya S. Turbanov, Aleksey A. Bolotovskiy, Alexander A. Gandlin, Boris A. Levin
Data type: docx
Explanation note: Additional material on *Phoxinus ayukensis* sp. nov. and comparative material on *Phoxinus adagumicus*, *P. chrysoprasius*, *P. colchicus* and *Phoxinus isetensis*.

Link: https://doi.org/10.3897/zse.101.153548.suppl6